JOURNAL OF CLINICAL MICROBIOLOGY, May 2000, p. 1860–1865 Vol. 38, No. 5
0095-1137/00/$04.00+0

Genetic Relatedness of *Salmonella* Isolates from Nondomestic Birds in Southeastern United States

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Received 30 November 1999/Returned for modification 8 January 2000/Accepted 1 March 2000

***Salmonella* infections have been implicated in large-scale die-offs of wild birds in the United States. Although we know quite a bit about the epidemiology of *Salmonella* infection among domestic fowl, we know little about the incidence, epidemiology, and genetic relatedness of salmonellae in nondomestic birds. To gain further insight into salmonellae in these hosts, 22 *Salmonella* isolates from diseased nondomestic birds were screened for the presence of virulence and antibiotic resistance-associated genes and compared genetically using pulsed-field gel electrophoresis (PFGE) and random amplified polymorphic DNA analysis. Of the 22 *Salmonella* isolates examined, 15 were positive for the invasion gene *invA* and the virulence plasmid-associated genes *spvC* and *pef*. Most (15 of 22) were generally sensitive to antibiotics. However, two *Salmonella* isolates from pet birds were identified as *Salmonella enterica* serovar Typhimurium DT104. Despite the general susceptibility of these *Salmonella* isolates to most antimicrobial agents, antibiotic resistance-associated genes *intI1*, *merA*, and *aadA1* were identified in a number of these isolates. Five distinct *Xba*I and nine distinct *Bln*I DNA patterns were observed for the 22 *Salmonella* isolates typed by PFGE. PFGE analysis determined that *Salmonella* isolates from passerines in Georgia and Wyoming were genetically related.**

Salmonella spp. can infect and cause significant disease in free-ranging and captive birds. Most salmonellosis in free-ranging wild birds is documented via case reports and other clinical observations (12, 18, 34, 41), with outbreaks in wildlife birds occurring during the winter months. While many outbreaks are localized, several large-scale epizootics have occurred. Recently, a widespread epizootic of salmonellosis was reported during the winter of 1997 to 1998 in free-ranging birds across the northeastern and midwestern United States (National Wildlife Health Center, unpublished data). Hundreds of birds, mostly songbirds, were reported to have died in this epizootic, yet little is known about the *Salmonella* spp. found in these free-ranging species. *Salmonella* spp. have been isolated from numerous free-ranging avian species, including passerine (18, 23, 25, 34, 36, 37, 41, 44, 59), psittacine (43, 45, 46), and gallinaceous birds (16, 19, 26); free-ranging and captive waterfowl (13, 33, 44, 49, 53); and raptors (6, 27, 28). Cases of salmonellosis in free-ranging birds, particularly in passerines, have been reported in several countries (25, 35, 37) and several eastern and midwestern states in the United States (18, 24, 34, 41) since 1957, when this disease was first documented in wild birds (24). The most common *Salmonella* serotype encountered is *Salmonella enterica* serovar Typhimurium (18, 24, 25, 34, 41, 59), a broad-host-range serotype that is commonly associated with cases of human salmonellosis in the United States (9). The high incidence of *S. enterica* serovar Typhimurium among passerines (47, 58) and their subsequent congregation around bird feeders may offer some opportunity for the transmission of this organism to humans.

Over the last decade, the Southeastern Cooperative Wildlife Disease Study has performed 264 bacterial cultures on over 50 species of free-ranging birds that were submitted for necropsy through the Southeastern Cooperative Wildlife Disease Study diagnostic service. Eighteen *Salmonella* isolates were obtained from those 264 bacterial cultures, giving a prevalence of 6.8%. Fourteen of the 18 cases were diagnosed as clinical salmonellosis, suggesting that these birds were not unapparent carriers. Nine of those 18 isolates were preserved, allowing this retrospective comparison of the *Salmonella* isolates found in free-ranging birds. Necropsy of these wild birds revealed that lesions associated with salmonellosis in passerine birds were distinctly different from the disease observed in other free-ranging and captive nondomestic birds. Is the unique pathology associated with salmonellosis in passerine birds, like goldfinches, due to a single *Salmonella* clone, or is the disease syndrome a function of the host species? To address the former question, we typed *Salmonella* isolates by pulsed-field gel electrophoresis (PFGE) and random amplified polymorphic DNA (RAPD) analysis. We further characterized these isolates as to the distribution of virulence and antibiotic resistance-associated genes. In this report, we identify a single *S. enterica* serovar Typhimurium genetic type commonly associated with disease in passerine species. Also, we identify the first reported case of multiple-drug-resistant *S. enterica* serovar Typhimurium DT104 from two psittacine companion birds, indicating a potential disease risk for both bird owners and fanciers in the United States.

MATERIALS AND METHODS

Bacterial isolates. All of the *Salmonella* isolates used in this study are listed in Table 1. *S. enterica* serovar Typhimurium SR11 and *Escherichia coli* HB101 were included in PCR and invasion assays as positive and negative controls.

Antimicrobial susceptibility determination. Antimicrobial MICs of *E. coli* isolates were determined via the Sensititre automated antimicrobial susceptibility system (Trek Diagnostic Systems, Westlake, Ohio) and interpreted according to the National Committee for Clinical Laboratory Standards guidelines for broth microdilution methods (39, 40). Sensititre susceptibility testing was per-

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TABLE 1. Gross and histologic lesions associated with salmonellosis in nondomestic birds

Species[a]	Isolate	Genetic type[b]		State	Gross lesions[c]			Histologic lesions[c]		
		*Bln*I	*Xba*I		UGI	LGI	Liver	UGI	LGI	Liver
Passerine birds										
Goldfinch (A, F)	98A-33516	B3	A2	Ky.	+	+	−	+	+	+
Cowbird (A, F)	98A-28238	B1-B	A1-B	Ga.	−	−	−	−	+	−
Cowbird (A, F)	98A-24966	B1-B	A1-B	Ga.	+	−	−	+	−	−
Cowbird (A, F)	96A-37518	B1-B	A1-B	Ga.	−	−	−	−	−	+
English sparrow (A, F)	98A-7582	B1-A	A1-A	Wy.	+	−	+	+	−	+
English sparrow (A, F)	98A-12202	B1-A	A1-A	Wy.	+	−	−	+	−	−
English sparrow (A, F)	98W-12198	B1-A	A1-A	Wy.	−	−	−	−	−	−
Gallinaceous birds										
Pheasant (A, F)	98A-6836	A	B	Wy.	−	−	−	−	−	−
Wild turkey (A, F)	96A-29192	C	C	W.V.	−	+	+			
Coturnix quail (A, F)	98A-9551	D	D	Ga.	−	−	−	−	−	+
Quail (A)	97-9746	D	D	Ga.	−	−	−	−	−	−
Psittacine birds										
Parakeet (C)	97A-9840	H	A2	Ga.	−	−	−	−	−	+
African grey parrot (C)	97A-47510	H	A3	N.C.	−	−	−	−	−	+
Senegal parrot (C)	98A-3397	F	F	Ga.	−	−	−	−	−	+
Lorikeet (C)	98A-3398	F	F	Ga.	−	−	−	−	−	−
Other species										
Owl (A, F)	96A-38567	B3	A3	Ga.	−	−	−	−	+	+
Heron (A, F)	97A-11201	E	A3	Ga.	−	−	+	+[d]	−	+
Emu (C)	97A-17868	B2	A2	Fla.	−	−	−	−	+	−
Emu (C)	98A-31777	I	E	Ga.	−	−	−	−	−	−
Laughing gull (A, F)	98A-17535	B2	A1-B	Fla.	−	−	−	−	+	−
Pigeon (A, F)	97A-26782	B3	H	N.J.	−	−	−	−	−	−
Hawk (F)	7992	G	G	Ga.	−	+	−	−		+

[a] *Salmonella* species were isolated from diseased birds. These nondomestic birds, examined at the Athens Diagnostic Laboratory, represent either free-ranging (F) or captive (C) birds. A, adult animals.

[b] PFGE was used to type *Salmonella* isolates. For PFGE, the restriction enzymes *Xba*I and *Bln*I were selected for typing of bacterial isolates. An alphabetical designation was assigned to each distinct DNA fingerprint.

[c] UGI, upper GI tract; LGI, lower GI tract.

[d] Lesions in proventriculus.

formed in accordance with the manufacturer's instructions. The following antimicrobials were assayed: amikacin, amoxicillin-clavulanic acid, ampicillin, ceftiofur, cephalothin, chloramphenicol, ciprofloxacin, gentamicin, kanamycin, nalidixic acid, streptomycin, sulfamethoxazole, tetracycline, and trimethoprim-sulfamethoxazole.

RAPD analysis. *Salmonella* isolates were typed by RAPD analysis using primer 1254 (23). RAPD PCR was performed using the Rapidcycler hot-air thermocycler (Idaho Technologies, Idaho Falls, Idaho) (60). The RAPD conditions used were those of Maurer et al. (36). DNA was separated on 1.5% agarose and 1× TAE gel with ethidium bromide (0.2 μg/ml) at 100 V for 1 h (50). The 100-bp ladder (GIBCO/BRL, Gaithersburg, Md.) was used as a molecular weight standard for determining the molecular weights of the PCR products.

PFGE. Agarose-embedded bacterial genomic DNA was digested with 10 U of restriction enzyme *Xba*I overnight at 37°C, and DNA fragments were separated by PFGE (3, 49) in a 1% agarose gel (Bio-Rad, Hercules, Calif.) using the CHEF DR-II electrophoretic apparatus (Bio-Rad). Electrophoresis was done for 25 h with a voltage of 200 V and a linearly ramped pulse time of 2 to 40 s (3). A lambda ladder (582 to 48.5 kb; Bio-Rad) served as the MW markers for PFGE. The restriction enzyme *Xba*I has proven useful in the typing of *Salmonella* (2, 42) and other gram-negative bacteria (3) by PFGE. For isolates with indistinguishable *Xba*I PFGE DNA patterns, RAPD analysis (23) and a second PFGE using *Bln*I as the restriction enzyme (57) were done to confirm their genetic relatedness.

Identification of *S. enterica* serovar Typhimurium DT104 multidrug resistance locus by PCR mapping. Since florfenicol resistance gene *flo* has been mapped to a site between two type 1 integrons in *S. enterica* serovar Typhimurium DT104 (11), PCR primers were designed to amplify a portion of *flo* and antibiotic resistance genes mapping upstream (*qac*Δ*E*) and downstream (*tetR*) of this gene (GenBank accession no. AF071555). Primers flomap1F (GATGTCTAACAAT TCGTTCAG) and flomap1R (CAACGTGAGTTGGATCATAG) anneal to positions 2751 of *qacDE* and 4609 of *flo*, respectively, to amplify a 1,858-bp DNA fragment. The second set of primers flomap2F (GGGATCGGCGAACTTTAC) and flomap2R (TGTGGTCGGTTCCGTTCTC), anneal at positions 5330 of *flo* and 6073 of *tetR* to produce a 744-bp amplicand by PCR. The PCRs were done using the Idaho Technologies Rapidcycler. The 10 PCR mixture consisted of 3 mM $MgCl_2$ (flomap1 primers) or 2 mM $MgCl_2$ (flomap2 primers), 50 mM Tris (pH 7.4), bovine serum albumin at 0.25 mg/ml, 0.1 mM primer, 0.2 mM deoxynucleoside triphosphates (Boehringer Mannheim; Indianapolis, Ind.), 0.5 U of *Taq* polymerase (Boehringer Mannheim), and 100 ng of the DNA template. The program parameters for the PCR using the flomap1 primers consisted of a hold at 94°C for 15 s; 30 cycles of 94°C for 15 s (denaturation), 40°C for 1 min (annealing), and 72°C for 1 min (extension) with a slope of 2; and a final extension at 72°C for 4 min. The PCR parameters for the flomap2 primers was 30 cycles of 94°C for 1 s (denaturation), 46°C for 1 s (annealing), and 72°C for 15 s at a slope of 2.

Detection of *Salmonella* virulence genes and antibiotic resistance-associated genes. Four different sets of primers for *Salmonella* virulence genes *invA* (54), *spvC* (54), *sefC* (7), and *pef* (7) were used to generate DNA probes by PCR as described by Dodson et al. (15). The conditions for Southern hybridization were followed as previously described (15, 50), with hybridization and washes carried out at 68°C. DNA probes for antibiotic resistance-associated genes *intI1* (5, 29), *aadA1* (5, 29), and *merA* (5, 32) were also generated by PCR. Conditions for PCR and Southern analysis were performed as described by Bass et al. (5).

***Salmonella* cell invasion assay.** Budgie abdominal tumor cells (BATCs) were used in the invasion assays with the salmonellae. This is a permanent avian epithelial cell line derived from an abdominal tumor in a parakeet (22). *Salmonella* invasion was done by the procedure of Henderson et al. (22). The invasion results were reported as a ratio of the bacterial count that invaded the BATCs divided by the inoculum concentration. These values were then normalized such that the positive control, *S. enterica* serovar Typhimurium SR11, represented 100% invasion. All invasion levels greater than 1% were considered to be positive for invasion of BATCs (15).

RESULTS AND DISCUSSION

Clinical syndrome associated with *Salmonella* infection of nondomestic birds. *Salmonella* isolates from free-ranging birds

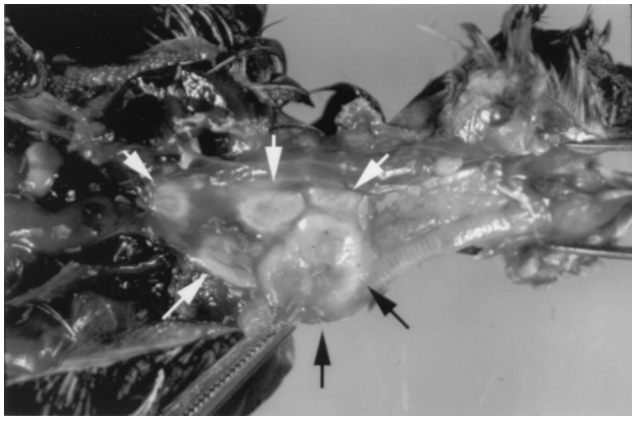

FIG. 1. Gross pathology of an American cowbird that died of salmonellosis. The arrows delineate multiple necrotic plaques on the esophageal mucosa from which *S. enterica* serovar Typhimurium was isolated.

were submitted from four southeastern states (Georgia, Florida, West Virginia, and Kentucky) and one western state (Wyoming). Five of the cases in wild birds were epizootics that resulted in morbidity and mortality of numerous birds. The four additional cases involving wild birds were individual animal submissions.

The lesions classically associated with salmonellosis in songbirds consist of necrotic plaques in the esophagus and crop (18, 25). In this study, this multifocal, necrotizing esophagitis and ingluvitis were only seen in passerine species (cowbirds, goldfinches, and English sparrows) (Fig. 1) and *S. enterica* serovar Typhimurium was isolated from the necrotic foci in all cases. Histologically, the epithelial surface was focally ulcerated, leaving a thick layer of necrotic cellular debris admixed with degenerate and intact leukocytes and myriads of gram-negative bacterial rods. The underlying submucosa had a focally intense infiltrate of heterophils and fewer lymphocytes and plasma cells. No evidence was found of other known pathogens, such as avian poxvirus, that can cause esophageal lesions in passerine species. *Trichomonas gallinae*, a flagellate protozoan that causes esophageal disease in columbiform species, such as mourning doves and, less commonly, predator birds, has not been reported in songbird species (9).

Among all of the examined bird species, the most consistent lesion reported at necropsy was emaciation but autolysis may have obscured subtle lesions in many of the cases (Table 1). Liver lesions, which were found grossly or histologically in 11 of the 22 cases, consisted of randomly scattered, small, pale foci that, histologically, were small foci of hepatocellular necrosis and inflammation. When present, lower gastrointestinal (GI) tract lesions consisted of acute hemorrhagic or necrotizing enteritis or enterocolitis. The caseous cecal plugs occasionally seen in domestic poultry were not found in these nondomestic birds. Other gross lesions less commonly encountered included small 1- to 2-mm caseous granulomas (found in the caudal airsacs of the quail and one cowbird). Other histologic lesions included orchitis and conjunctivitis (both diagnosed in cowbirds), meningitis (diagnosed in the pigeon), and septicemia (diagnosed in a cowbird, the African grey parrot, and the Senegal parrot).

GI lesions are common in the lower GI tract in poultry (20). However, the necrotic esophageal plaques seen in passerine birds have not been reported in domesticated gallinaceous birds. Disease was generally reported for adult birds, which contrasts with salmonellosis in domestic poultry where illness is generally observed in younger (1- to 7-day-old) birds (20). The *Salmonella* isolates from these diseased birds were genetically typed to determine if there was a pathogenic clone associated with this illness in passerine birds.

Genetic diversity among *Salmonella* strains associated with nondomestic birds. Five distinct *Xba*I DNA patterns were observed for the 23 *Salmonella* isolates typed by PFGE (Table 2). Isolates with seven or more different DNA fragments were assigned to a specific PFGE *Xba*I genetic type, A to E, as recommended by Tenover et al. (55). The majority (70%) of these *Salmonella* isolates belonged to PFGE *Xba*I genetic type A. Within this PFGE group, there were additional differences of four to six DNA fragments within PFGE pattern A to further differentiate these related *Salmonella* isolates into three subgroupings, A1 to A3. A group of closely related isolates were identified that differed by only two or three DNA fragments to justify a final subcategory, $A1_{A\text{-}B}$. Using a second restriction enzyme, *Bln*I, we were able to distinguish among *Salmonella* isolates by PFGE that produced similar or identical PFGE patterns, earlier, with *Xba*I (Table 2). PFGE using both restriction enzymes *Xba*I and *Bln*I identified nine distinct genetic types among *Salmonella* isolates from nondomestic birds. However, half of these isolates appeared to be possibly related, according to their PFGE pattern. Five distinct genetic types were identified among the *Salmonella* isolates from Georgia. Four of these five *Salmonella* genetic types were found only in that state. Likewise, other *Salmonella* genetic types, as defined by PFGE, appeared to be unique to other locales from which these specimens were obtained. Certain *Salmonella* genetic types therefore appear to be endemic.

There are other genetic types, however, that do not appear to be geographically restricted. For example, closely related *Salmonella* genetic types were identified by PFGE among isolates obtained from Georgia and Wyoming. We were unable to definitively separate *Salmonella* isolates A7582, 98A-24966-2, and 98-28238 further using another molecular typing tool, RAPD PCR (Table 2). These genetically related salmonellae were all isolated from passerines.

We also noted that *Salmonella* isolates from the Lorikeet and Senegal parrot were indistinguishable by their PFGE and RAPD patterns as well. Both birds were from the same pet owner. Since *Salmonella* isolates from the lorikeet and the Senegal parrot had a pentadrug resistance pattern similar to that of *S. enterica* serovar Typhimurium DT104 (21), we compared their PFGE patterns. *S. enterica* serovar Typhimurium DT104 isolates are genetically related, producing PFGE patterns that are indistinguishable (1). We noted that our *Salmonella* isolates had PFGE patterns indistinguishable from the DNA fingerprint of *S. enterica* serovar Typhimurium DT104 (data not shown). In addition to similarities in PFGE patterns, these *Salmonella* isolates were similar to *S. enterica* serovar Typhimurium DT104 with regard to the genetic organization of the multidrug resistance locus (11). The *Salmonella* isolates from the lorikeet and the Senegal parrot had the florfenicol resistance gene *flo*, a marker for multidrug-resistant *S. enterica* serovar Typhimurium DT104 (10). In these isolates, the florfenicol resistance gene *flo* is flanked by quaternary ammonium resistance gene *qac*Δ*E* and tetracycline resistance gene *tetR*. PCR analysis revealed that the psittacine *Salmonella* isolates had an arrangement of antibiotic resistance genes similar to that of the *S. enterica* serovar Typhimurium DT104 multidrug resistance locus. Phage typing confirmed that these isolates were *S. enterica* serovar Typhimurium DT104.

Antimicrobial susceptibility and drug resistance genes present in *Salmonella* isolates from nondomestic birds. Most of the *Salmonella* isolates from nondomestic birds (15 of 22) in

TABLE 2. *Salmonella* isolates used in this study

Isolate	State	Source[a]	Serovar	% Invasion[b]	Antibiotic resistance[c]	Antibiotic resistance-associated gene[d]			Virulence-associated gene[e]			Genetic type[f]		
						intI1	*merA*	*aadA1*	*invA*	*spvB*	*pef*	PFGE (*Bln*I)	PFGE (*Xba*I)	RAPD (1254)
98A-6836	Wy.	Pheasant (C)	Infantis	ND		−	−	−	+	−	−	A	B	BB
98A-7582	Wy.	English sparrow (F)	Typhimurium	ND		+	−	−	+	+	+	B1-A	A1-A	DD
98A-12202	Wy.	English sparrow (F)	Typhimurium	ND		−	−	−	+	+	+	B1-A	A1-A	FF
98W-12198	Wy.	English sparrow (F)	Typhimurium	ND		−	−	−	+	+	+	B1-A	A1-A	DD
96A-29192	W.V.	Wild turkey (F)	Java	100		+	−	−	+	−	−	C	C	CC
96A-37518	Ga.	Cowbird (F)	Typhimurium	ND		−	−	−	+	+	+	B1-B	A1-B	DD
96A-38567	Ga.	Owl (F)	4,5,12:I-monophasic	8	Su Tc	+	+	−	+	+	+	B3	A3	EE
97-9746	Ga.	Quail	Typhimurium (Copenhagen)	ND	Ap Kn Sm Su Tc	+	+	+	+	+	−	D	D	DD
97A-9840	Ga.	Parakeet (C)	Typhimurium	25		−	−	−	+	+	+	H	A2	DD
97A-11201	Ga.	Heron (F)	4,5,12:I-monophasic	125		−	−	−	+	+	+	E	A3	DD
97A-17868	Fla.	Emu (C)	Typhimurium	175		−	−	−	+	−	−	B2	A2	DD
97A-47510	N.C.	African grey parrot (C)	Typhimurium	ND	Su	−	−	−	+	+	+	H	A3	DD
98A-9551	Ga.	Coturnix quail (F)	Typhimurium (Copenhagen)	105	Ap Kn Sm Su	+	−	+	+	+	−	D	D	DD
98A-17535	Fla.	Laughing gull (F)	Typhimurium	140		−	−	−	+	+	+	B2	A1-B	AA
98A-24966-2	Ga.	Cowbird (F)	Typhimurium	50		+	−	−	+	+	+	B1-B	A1-B	DD
98A-28238	Ga.	Cowbird (F)	Typhimurium	65		+	−	−	+	+	+	B1-B	A1-B	DD
98A-33516	Ky.	Goldfinch (F)	Typhimurium (Copenhagen)	ND	Su	−	−	−	+	−	−	B3	A2	DD
98A-3397	Ga.	Senegal parrot (C)	Typhimurium (Copenhagen) DT104	ND	Ap Cm Sm Su Tc	+	−	+	+	+	+	F	F	DD
98A-3398	Ga.	Lorikeet (C)	Typhimurium (Copenhagen) DT104	ND	Ap Cm Sm Su Tc	+	−	+	+	+	+	F	F	DD
7992	Ga.	Hawk (F)	Alabama	ND		−	−	−	+	−	−	G	G	GG
98A-31777	Ga.	Emu (C)	Anatum	ND		−	−	−	+	−	−	I	E	HH
97A-26782	N.J.	Pigeon (F)	Typhimurium (Copenhagen)	ND		−	−	−	+	+	+	B3	H	DD

[a] *Salmonella* isolates were from diseased birds. These nondomestic birds, examined at the Athens Diagnostic Laboratory, represent either free-ranging (F) or captive (C) birds.

[b] Invasion is presented as a percentage relative to levels obtained with *Salmonella enterica* serovar Typhimurium SR11. *E. coli* K-12 strain HB101 was included as an invasion-negative control for BATCs (21).

[c] Abbreviations: Ap, ampicillin; Cm, chloramphenicol; Kn, kanamycin; Sm, streptomycin; Su, sulfamethoxazole; Tc, tetracycline. All isolates were sensitive to amikacin, apramycin, ceftiofur, ceftriaxone, cephalothin, ciprofloxacin, gentamicin, nalidixic acid, and trimethoprim.

[d] *Salmonella* isolates were screened by DNA-DNA hybridization for the presence of class 1 integrons and resistance genes associated with them. *intI1*, class 1 integrase; *merA*, mercury reductase; *aadA1*, streptomycin and spectinomycin resistance gene.

[e] *Salmonella* isolates were screened by DNA-DNA hybridization for the presence of invasion gene *invA* and virulence plasmid-associated genes *spvC* and *pef*.

[f] We chose PFGE and RAPD PCR for typing of *Salmonella* isolates. For PFGE, the restriction enzymes *Xba*I and *Bln*I were selected for typing of bacterial isolates. RAPD involved using a 10-mer oligonucleotide, 1254, as the typing primer. An alphabetical designation was assigned to each distinct DNA fingerprint.

TABLE 3. *Salmonella* genotypes

Phenotype and genotype	No. of isolates positive[a]
Antibiotic resistance	
intI1 alone	4
intI1 aadA1	3
intI1 aadA1 merA	1
intI1 merA	1
At least *intI1* gene	9
No antibiotic resistance-associated genes	12
Virulence	
invA alone	6
invA spvC	2
invA spvC pef	15

[a] Of a total of 22 isolates.

this study were sensitive to 16 antibiotics. All of the isolates, including multidrug-resistant *Salmonella* isolates 98A-3397, 98-3398, 97-9746, and 98A-9551, were sensitive to amikacin, apramycin, ceftiofur, ceftriaxone, cephalothin, ciprofloxacin, gentamicin, nalidixic acid, and trimethoprim. Seven of the 22 *Salmonella* isolates were resistant to sulfamethoxazole (MIC, >512 μg/ml), and of the seven sulfamethoxazole-resistant *Salmonella* isolates, four were also resistant to streptomycin (MICs, 64 to >256 μg/ml). We also observed resistance to other drugs, including ampicillin (MIC, >32 μg/ml), tetracycline (MIC, ≥32 μg/ml), chloramphenicol (MIC, >32 μg/ml), and kanamycin (MIC, >64 μg/ml). With one exception, *Salmonella* isolates from passerine birds were sensitive to antibiotics while those from quail and psittacines were resistant to one or more antibiotics. The drug resistance patterns and percentages were clearly different in *Salmonella* isolates cultured from nondomestic birds compared to those from poultry (38). In addition to being resistant to sulfamethoxazole and streptomycin, the poultry *Salmonella* isolates are also resistant to tetracyclines and gentamicin (38).

Rapid dissemination of drug resistance is often attributed to integrons, genetic elements involved in swapping or combining of antibiotic resistance into the integration site (52). Class 1 integrons, and resistance genes associated with them, are widely distributed in nature. In our analysis of *Salmonella* isolates from nondomestic birds, 9 of the 22 isolates contained the integrase, *intI1*, gene, a marker for class 1 integrons (Table 3). In addition to *intI1*, *Salmonella* isolates also contained other markers associated with class 1 integrons, including the streptomycin-spectinomycin resistance gene *aadA1* and the mercuric reductase gene *merA* (31), but at a lower frequency than *intI1*. Twelve of the 22 *Salmonella* isolates did not have *intI1* or the other antibiotic resistance-associated genes, *aadA1* and *merA*.

Virulence potential of *Salmonella* isolates from nondomestic birds. *Salmonella* pathogenesis is dictated by a series of genes responsible for colonization (56), invasion (48), and spread (4, 30) within its avian host. Host adaptation is influenced by the distribution of fimbrial and nonfimbrial adhesins among the salmonellae (7). To assess potential virulence of *Salmonella* isolates by the presence or absence of genes, PCR and Southern hybridization were used to detect *Salmonella* virulence genes. All of the *Salmonella* isolates contained the invasion gene *invA*. Most of the *Salmonella* isolates (17 of 22) from diseased nondomestic birds also contained the virulence plasmid, as is evident from the incidence of *spvC* among these isolates (Table 3). Of the 17 *Salmonella* isolates positive for *spvC*, 15 also contained the *Salmonella* plasmid-encoded fimbrial gene *pef* (7). *pef* appears to be restricted in its distribution to a few *Salmonella* serotypes, Typhimurium, Paratyphi, Choleraesuis, and Typhi (7). All of the *Salmonella* isolates were negative for *sefC* (data not shown), a fimbrial gene found primarily in the avian-adapted salmonellae, *S. enterica* subsp. *enteritidis*, *S. enterica* subsp. *pullorum*, and *S. enterica* subsp. *gallinarum* (7, 14, 15). The *S. enterica* serovar Typhimurium isolates from free-ranging birds do not appear to be any different from the broad-host-range *S. enterica* serovar Typhimurium strains associated with other avian and mammalian hosts with regard to the distribution of adhesion (7) and invasion (54) genes.

We also examined these *Salmonella* isolates for phenotypic differences in the ability to invade epithelial cells. All of the *Salmonella* isolates were considered to be invasive for avian epithelial cells, with levels ranging from 8 to 175% invasion compared to the control, *S. enterica* serovar Typhimurium SR11 (Table 2). These *Salmonella* isolates were more efficient at invading avian epithelial cells than avian host-specific *S. enterica* subsp. *pullorum* (15). Variability in the invasion phenotype did not correlate with a specific genetic type, as defined by PFGE pattern, or virulence genotype.

According to genotype and antibiotic resistance pattern, the *Salmonella* strains associated with captive and free-ranging nondomestic birds represent a potential threat to humans. Most of the *Salmonella* isolates possessed the 90-kb virulence plasmid, enabling the organisms to colonize and spread in an avian or nonavian host. The other troubling news is the identification of multidrug-resistant *S. enterica* serovar Typhimurium DT104 from pet birds. As far as we are aware, this is the first reported case of *Salmonella* DT104 from exotic birds in the United States. Nondomestic birds can obviously serve as a reservoir for transmission of salmonellae to pet owners and bird watchers. Bird feeders are popular in the United States, and determining the incidence of salmonellae at these feeders is important in order to assess their health risk to the general population.

ACKNOWLEDGMENTS

This research was funded by a grant from the U.S. Poultry and Egg Association, the State of Georgia's Veterinary Medicine Agricultural Research Grant, and USDA Formula Funds.

We thank Stanley Vezey and Susan Little for their comments and critique of the manuscript. We also thank Sherry Ayers for her technical assistance. Thanks to Elizabeth Williams of the Wyoming State Veterinary Diagnostic Laboratory for providing the four western *Salmonella* isolates.

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